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Discovery of Peptide-Based Antitoxins against Neurotoxins from Green and Black Mamba (*Dendroaspis* Family)

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Snakebite – A neglected threat to public health

Globally, more than 5.5 million people are bitten by venomous snakes every year, leading to an estimated 125,000 deaths and 3 times as many amputations [1,2,3]. The problem is most prevalent in Sub-Saharan Africa where affordability of antivenom is low, resulting in only 2% of snakebite victims receiving treatment [1,4]. Since the introduction of antivenoms in the 1890's, only modest advances in antivenom technology and production have been made. Current antivenoms are, therefore, still being produced by immunisation of large ruminants, typically horses, with snake venoms and subsequently bleeding them to collect blood comprising venom-specific antibodies [4]. The incompatibility of these antivenoms with the human immune system can lead to serious adverse effects [1,5]. A novel approach is needed in order to introduce safer, cheaper and more efficacious antivenoms that are compatible with the human immune system to the market.



Figure 1: *Dendroaspis polylepis* (black mamba). Photo: Larsa D. 2011

We attempt to discover cross-reactive, peptide-based antitoxins against the structurally similar dendrotoxins α -dendrotoxin (α -Dtx, UniProtKB P00980), isolated from *Dendroaspis angusticeps* (Green mamba), and dendrotoxin I (Dtx I, UniProtKB P00979) from *Dendroaspis polylepis* (Black mamba) by phage display [5,6]. Cross-reactive antitoxins with the ability to neutralise several toxins are of interest to antivenom development, since only a few cross-reactive antitoxins would be needed to neutralise a complete snake venom [4].

Method – Identification of binders with phage display

Initially, sequence alignment using the protein Needleman-Wunsch algorithm from EMBL-EBI was performed (Figure 3). Additionally, 3D structural models of the two toxins were constructed and compared. The structure of α -Dtx was based upon the available X-ray crystallographic structure with PDB entry 1DTX whilst the structure of Dtx I was estimated based on a model of the Kunitz-type serine protease inhibitor (PDB entry: 3BYB), isolated from *Pseudonaja textilis* (brown snake) using the Bioinformatics Toolkit developed by the Max-Planck Institute, Tübingen (Figure 3).

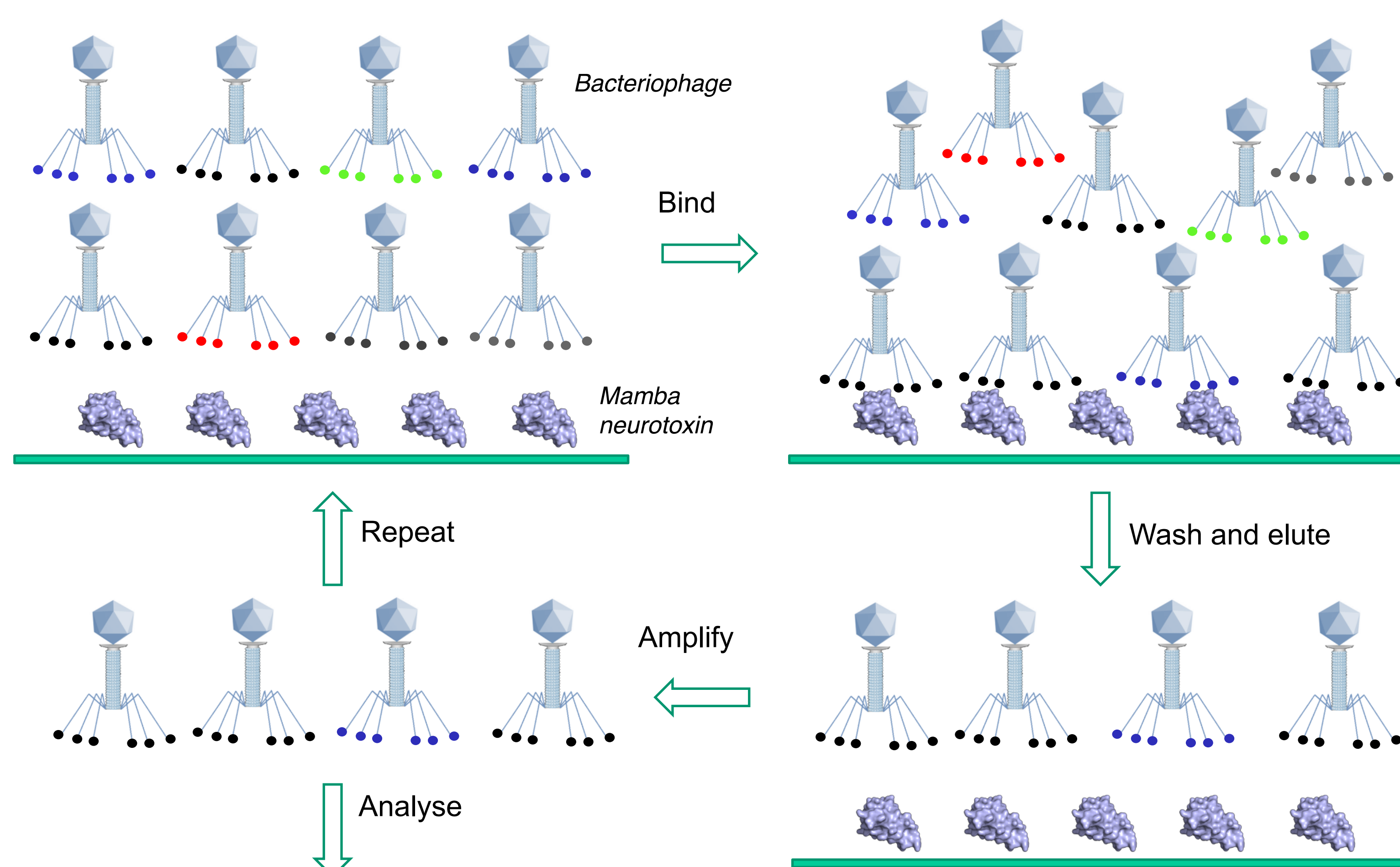


Figure 2: Phage display is a screening technique whereby peptides are displayed on the surface of bacteriophages, some of which bind with high affinity to snake toxins that are attached to plate wells.

Phage display was applied to discover binders to Dtx I and α -Dtx (Figure 2). Phage display is a screening technique, where a library of peptides is engineered to be expressed on the surface of bacteriophages by genetic fusion with a phage coat protein [7]. In order to identify specific binders, the mamba snake neurotoxins are attached to plate wells (Reacti-Bind™ plate) and, subsequently, the phage library is added to the wells. Phages that display a relevant peptide on their coat will be retained whilst unbound phages will be washed away. Iterative rounds of binding result in amplification of the phages expressing high-affinity peptides [7].

Results – Cross-reactivity based on similarity

Based on ELISA, strong binding to Dtx I was observed for the polyclonal phage library after the third round of panning (Figure 4), yet randomly selected monoclonal phages did not show strong binding to Dtx I (Figure 5). It was observed that the polyclonal phage library also bound to α -Dtx (data not shown), indicating a high degree of cross-reactivity. This was anticipated by the bioinformatics modelling of the dendrotoxins, illustrating a high degree of similarity in both their primary, secondary, and tertiary structures (Figure 3).

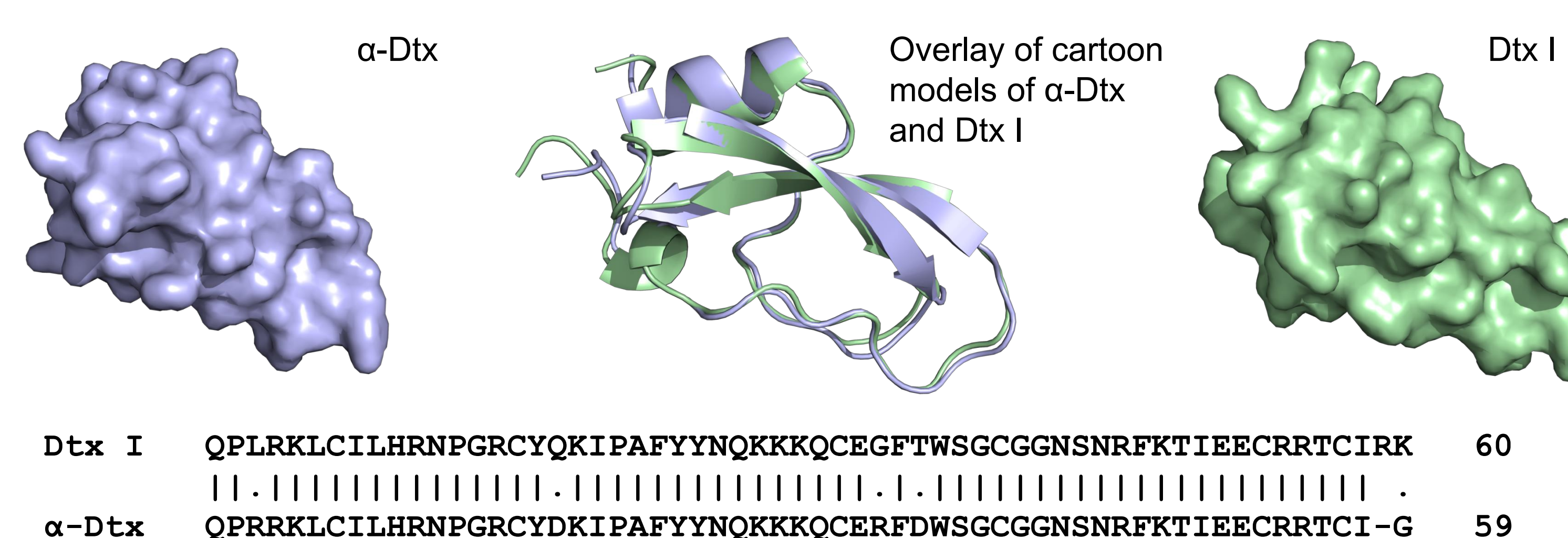


Figure 3: Surface models and overlapping cartoon models of α -dendrotoxin (α -Dtx) and dendrotoxin I (Dtx I) from *D. angusticeps* and *D. polylepis*, respectively, illustrating the largely similar secondary and tertiary structures of the two toxins. Models are drawn in PyMOL. Sequence similarity of 90% is observed when performing sequence alignment using the protein Needleman-Wunsch algorithm from EMBL-EBI.

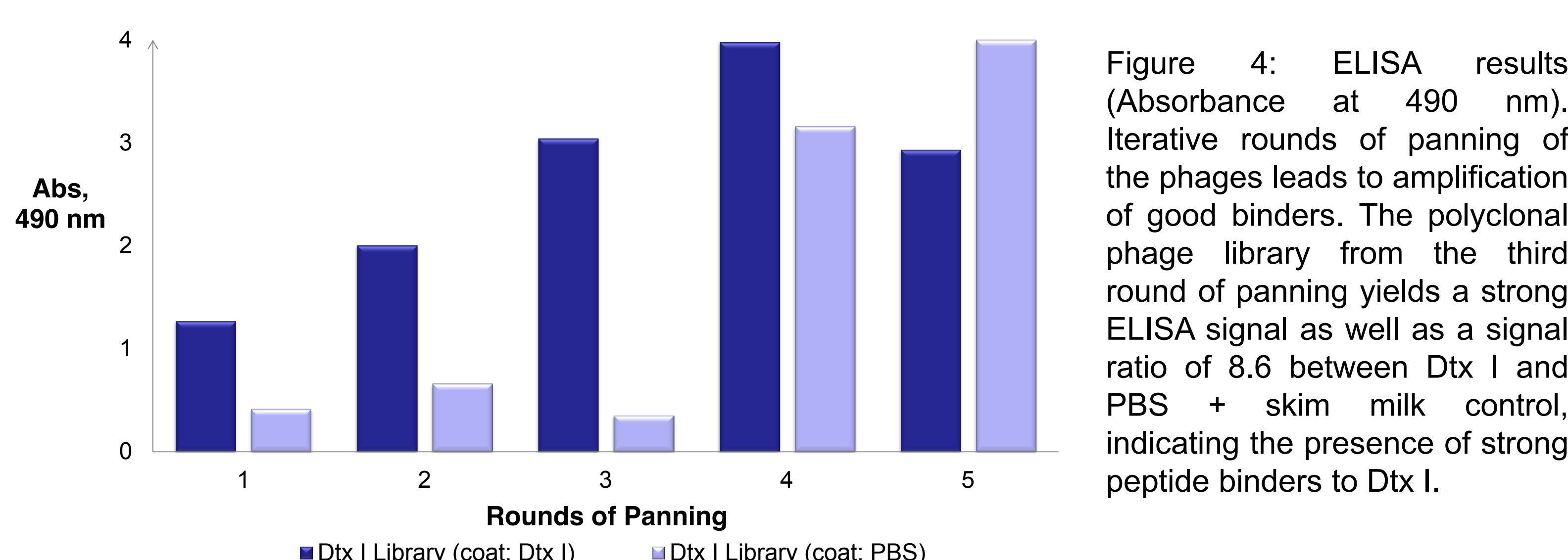


Figure 4: ELISA results (Absorbance at 490 nm). Iterative rounds of panning of the phages leads to amplification of good binders. The polyclonal phage library from the third round of panning yields a strong ELISA signal as well as a signal ratio of 8.6 between Dtx I and PBS + skim milk control, indicating the presence of strong peptide binders to Dtx I.

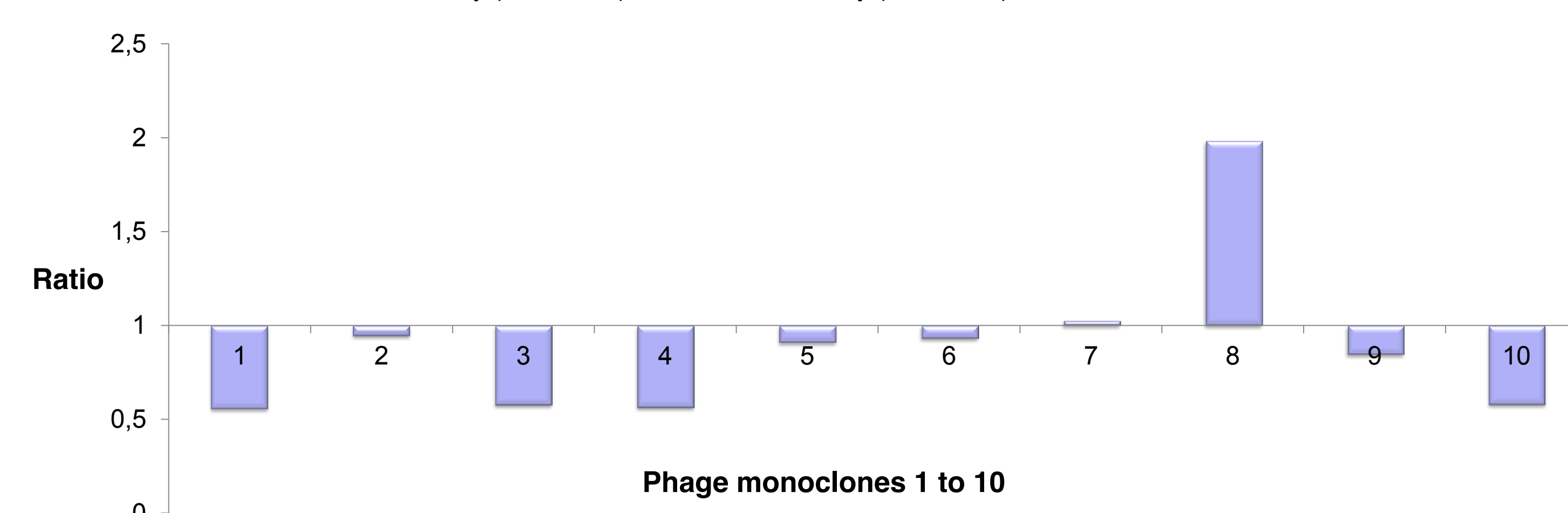


Figure 5: ELISA results (Absorbance at 490 nm) showing the signal ratio between binding to Dtx I and the PBS + skim milk control for 10 selected phage monoclonal clones from the third round of panning. An additional 20 phage monoclonal clones were selected and tested for binding to Dtx I (data not shown); however, better binders were not observed. The best binder is Phage Monoclonal 8, which shows some degree of binding, but only a specificity of 2:1 for Dtx I vs. the PBS + skim milk control.

Outlook – Discovery of antitoxins for mamba toxins

Polyclonal phages with strong binding affinity, high specificity, yet displaying cross-reactivity, were discovered using phage display. However, due to time limitations, no individual monoclonal phage was found to have both high affinity and show selectivity towards the toxins.

Subsequent steps could include further analysis of other monoclonal phages or repetition of the fourth round of panning in order to attempt to amplify phages with high affinity and specificity. If a high-affinity toxin binder were to be identified, this binder could 1) be applied as a peptide-based antitoxin, 2) be used to create a peptidomimetic antitoxin or 3) be grafted onto an antibody as a CDR region, paving the way for safer and more efficacious antivenoms.

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